

# **Characterization of the Structural and Chemical Properties of Copper Chelators in Seawater**

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## **LONG-TERM GOALS**

The long-term goal is to obtain a comprehensive understanding of copper behavior in harbors, from a chemical and biological perspective. This means understanding the relationship between chemical transformations and biological effects for copper derived from natural and anthropogenic sources. Results can be used by dischargers, like the US Navy, and regulators, including the EPA and local agencies, to make informed decisions about managing Cu inputs into harbors and other receiving waters.

## **OBJECTIVES**

The objectives of this project are to learn more about the chemical properties of copper binding ligands. Our work, and that of others, shows that these ligands control the variability in Cu bioavailability in many coastal waters. Current titration methodologies provide information about binding constants and concentrations only. Structural information is necessary to validate hypotheses about sources and sinks (with a view to modeling variability) and to identify compounds with unique properties of relevance to the navy.

## **APPROACH**

The approach has been to focus on chelators produced by ubiquitous marine phytoplankton and bacteria, rather than chelators actually in the water column, because they can be produced at much higher concentrations. In the past, we have made the case that some organisms may be important sources, such as the marine cyanobacterium *Synechococcus*. Cu stressed *Synechococcus* cultures produce a chelator with binding characteristics similar to the strongest ligands found in the water column.

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We have combined polarographic measurements of the ligand's concentration and thermodynamic properties, with a separation scheme to isolate the ligand for HPLC analysis.

## WORK COMPLETED

Protocols for optimizing production of *Synechococcus* chelators have been developed by studying ligand production rates as a function of growth rates. Our protocol results in ligand production at levels 100x greater than levels of the strongest ligands in natural waters.

Additional characterization of the ligand has been performed by pseudopolarography, enabling us to determine half-wave potentials for these complexes as a probe for redox state of the metal and thermodynamic binding constant.

We developed a scheme to isolate the ligands from seawater, as follows. Cultures were filtered, and the solution pH was adjusted to 3, followed by extraction into a 50:50 mixture of chloroform phenol. The ligands were back extracted into distilled water at pH 8, and the sample concentrated by freeze drying. Then the sample was purified chromatographically. Several columns and solvent gradients were evaluated. Fluorescence detection was utilized, and isolates collected by fraction collector for polarographic analysis of binding characteristics. Approximately 30 isolates were obtained, of which 4 have been analyzed by electrospray mass spectrometry. ICP-MS analyses were performed using the Finnegan system acquired by Nelson Frew with DURIP funds.

We have developed cultures of a freshwater *Synechococcus* to produce chelators. The objective is to produce chelators in a low ionic strength media to avoid salt interferences that have been an obstacle to MS analysis of many of our isolates (see below).

## RESULTS

Ligand production by these compounds is maximized when the algae are growing exponentially. For *Synechococcus*, ligand production is negligible when the algae are in stationary phase, suggesting that the ligand is produced by active metabolism, not a product of senescence or lysis.

Pseudopolarogram data show that the complexes produced by *Synechococcus* have a half-wave potential of  $-1$  V, suggesting that the thermodynamic binding constant is approximately  $10^{38}$ . This is extremely high, but if the compound is a Cu(I) complex the binding constant would be half that value- quite plausible for a complex with a cysteine-containing peptide. Adsorptive cathodic stripping voltammetry reveals a peak at  $-0.65$  V consistent with a thiol such as glutathione.

Mass spectral analysis has been difficult because of high residual salts in many primary isolates, and lack of signal in many of the more highly purified fractions. The best chromatographic separations have been obtained for demetallated samples, but the free

ligands appear to degrade and/or stick to the columns non-reversibly. This problem could reflect oxidation of thiols to disulfides.

Currently, we are culturing a freshwater *Synechococcus* to obtain primary isolates without any salts. We want to develop reliable protocols for the analysis of these samples before analyzing our remaining marine isolates, which required months of work. We are also investigating the molecular biology of Cu-regulated ligand production by *Synechococcus*. Eric Webb, a new WHOI postdoctoral scholar, is working on this project.

## **IMPACT/APPLICATIONS**

Successful characterization of this material could lead to new insight into the sources and chemistry of Cu ligands in seawater. Potential applications could arise if we identify a new class of chelators selective for Cu that could be used in remediation.

## **TRANSITIONS**

In Moffett's prior ONR work, an in situ sampling device was evaluated to study Cu bioavailability in dynamic environments. Moffett travelled to the naval base at Bremmerton (Wash.) to investigate the application of these devices with personnel there. He has also disseminated his work amongst the regulatory community, most recently at the EPA laboratory in Narragansett, R.I., where the original research leading to Cu criteria were developed.

## **RELATED PROJECTS**

Moffett is collaborating with Brian Palenik(Scripps) on a project to study the relationship between Cu chemistry in seawater and the production of a highly Cu specific cell-surface binding protein by marine diatoms. Palenik has developed an antibody to this protein which may be an excellent in situ indicator of metal stress.

## **PUBLICATIONS**

Submitted

Croot, P.L., Moffett, J.W. and Brand, L.E. Production of extracellular Cu chelators by eucaryotic phytoplankton in response to Cu stress. *Limnol. Oceanogr.*

In press

Bruland, K.W., E.L. Rue, J.R. Donat, S.A. Skrabal and J.W. Moffett. Intercomparison of voltammetric techniques to determine the chemical speciation of dissolved copper in a coastal seawater sample. *Anal. Chim Acta*

Croot, P.L. , Moffett, J.W. and Luther, G.W. Polarographic determination of the half wave potentials of copper complexes in seawater. *Marine Chemistry*